

Structural Changes of Cellulose Crystals during the Reversible Transformation Cellulose I $\rightleftharpoons$ III_I in *Valonia*

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Dedicated to D.A.I. Goring on the occasion of his retirement

Keywords

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Summary

The reversible swelling of cellulose I $\rightleftharpoons$ cellulose III_I was investigated by transmission electron microscopy using cross sections of *Valonia* cell wall swollen in ethylenediamine. By using diffraction contrast techniques, it was possible to visualize directly the cross-sectional shape of the cellulose microfibrils and the crystalline domains. The microfibrils sections were nearly square and regular in the initial sample, but became distorted and convoluted in *Valonia* III_I and regenerated *Valonia* I. At the crystalline level, the conversion from *Valonia* I to *Valonia* III_I involved an extensive decrystallization and fragmentation of the cellulose crystals. During the conversion back to cellulose I, partial recrystallization took place but the distortion and fragmentation of the crystals were irreversible. The transformation was also monitored by electron diffraction analysis. This technique showed that the unique uniplanar-axial orientation of the crystalline cellulose microfibrils was irreversibly lost during the swelling and washing steps leading to cellulose III_I.

Introduction

The study of the polymorphism of cellulose is of great scientific and technological importance. In its native state, cellulose is microfibrillar and exhibits the crystalline form called cellulose I. This unique structure is commonly thought to result from the combined action of biosynthesis and crystallization. In its solid state, cellulose I can be converted into other crystalline forms, namely cellulose II, cellulose III and cellulose IV. Each of these polymorphs can be identified by its x-ray diagram which denotes a specific lateral packing of the cellulose chains within their unit cell (Marchessault and Sarko 1967; Ellefsen and Tønnesen 1971; Sarko 1976).

The solid state conversion of cellulose I into cellulose II, the so-called mercerization process, is normally achieved by swelling native cellulose in a concen-

trated alkali solution, followed by subsequent washing with water. The mercerization is considered to be irreversible since, up to now, it has not been possible to find a procedure where cellulose II could be converted back into cellulose I. During mercerization, the microfibrillar nature of native cellulose is strongly affected and even in some cases, partially lost: this denotes a profound modification of the supramolecular arrangement of cellulose during the process.

A different situation prevails in the case of cellulose III. The intra-crystalline swelling of either cellulose I or cellulose II with liquid ammonia (Barry *et al.* 1936) or a variety of amines (Davis *et al.* 1943) or polyamines (Creely and Wade 1978), followed by the removal of the swelling agent, produces cellulose III_I or cellulose III_{II} respectively. As opposed to the mercerization process, the formation of cellulose III is reversible since a hot water treatment is normally sufficient to convert this polymorph back into its original state.

The reversibility of the solid state transformation: cellulose I $\rightleftharpoons$ cellulose III_I has been investigated in some

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detail because such a conversion process stands out as among the best to increase the accessibility and reactivity of native cellulose (Herrick 1983) without losing its fibrous characteristics. It is generally accepted that the reversibility of the transformation occurs only at the cellulose unit cell level, but that there is a considerable amount of disorganization at the ultrastructural level, since a great number of crystalline defects are created when cellulose has been treated by ammonia of various amines. At present, it is not certain whether these defects are created during the intracrystalline swelling of cellulose or during the subsequent de-swelling step, leading to cellulose III_I.

In a previous study (Roche and Chanzy 1981), we focussed on the ultrastructural aspect of the transformation cellulose I $\rightleftharpoons$ cellulose III_I in *Valonia*. The observations, in particular those made by dark field transmission electron microscopy (TEM), revealed fracturing and longitudinal splitting of the initial *Valonia* fibrillar crystals during the transformation. The occurrence of such crystalline defects was permanent. In particular, they could not be eliminated when the reverse transformation from cellulose III_I to cellulose I was effected.

Subsequent to our previous study, recent advances have been made in high resolution observations of cellulose samples by TEM. In particular, well resolved diffraction contrast images have been obtained recently on ultra-thin transverse sections of native cellulose microfibrils from *Valonia* (Revol 1982; Sugiyama *et al.* 1985). These images show that the microfibrils are perfect crystals with a nearly square cross-section. In this present work, the re-examination of the fibrous transformation: cellulose I $\rightleftharpoons$ cellulose III_I was undertaken by using these improved TEM techniques on ultrathin cross-sections of untreated and amine treated *Valonia* cell walls. Electron diffraction analysis was also performed to corroborate the electron micrographs results.

Materials and Methods

Valonia ventricosa vesicles were harvested from the sea bed in the Lower Keys, Florida. The cells were slit open, emptied and the cell walls were allowed to dry by pressing them between two sheets of filter paper. When needed, the dried walls were purified from non cellulosic contaminants according to the procedure described by Gardner and Blackwell (1974). They were then stored in methanol.

The transformation *Valonia* I $\rightarrow$ *Valonia* III_I was achieved by immersing fragments of purified cell wall in anhydrous ethylenediamine for one hour. This treatment, which was followed by washing in methanol for 15 minutes, was repeated seven times in order to achieve total conversion into *Valonia* III_I. The reverse transformation *Valonia* III_I $\rightarrow$ regenerated *Valonia* I, was performed by hydrothermal treatment of *Valonia* III_I at 160°C for 60 minutes inside a sealed pressure vessel.

Flat fragments of initial *Valonia* I, *Valonia* III_I and regenerated *Valonia* I were embedded in low viscosity spurr resin. The resulting blocks were examined with a polarizing optical microscope be-

tween crossed-nicols. This enabled us to identify the two main orientations of the cellulose microfibrils within a given flat area of the embedded fragments of *Valonia* cell wall. Ultrathin sections were then made with a LKB OMU 3 ultramicrotome equipped with a diamond knife, the sections being cut perpendicular to one of the two main orientations of the microfibrils.

The ultrathin sections were observed with a Philips EM400T TEM, operating at 120 kV, in bright field diffraction contrast conditions (objective aperture of 20 microns) with low dose illumination. Pictures of a previously unirradiated area of the specimen were recorded at a magnification of 17,000 X – 28,000 X using Ilford films (Ilford, France). Selected-area-electron diffraction patterns were obtained by using a micro-beam diffraction procedure (Thomas 1984).

Results

Electron micrographs of the cross sections of the three samples under investigation, namely *Valonia* I, *Valonia* III_I and regenerated *Valonia* I, are presented in Figures 1 and 2. These images were all recorded using diffraction contrast in the bright field mode and are printed in direct contrast in Figures 1A, 1B, 1C and in reverse contrast in Figures 2A, 2B, 2C which are enlargements of Figures 1A, 1B, 1C respectively. Figure 1 shows the well known (Preston 1974) lamellation of crisscrossed layers of cellulose microfibrils. The lamellar arrangement is maintained in the three samples; the stacking of the lamellae however is tight in *Valonia* I and *Valonia* III_I but much looser in regenerated *Valonia* I.

Figure 1A corresponds to the initial sample and is similar to the diffraction contrast images already reported for the cross-sections of *Valonia* cell walls (Revol 1982, Sugiyama *et al.* 1985). The area indicated by the arrow corresponds to a layer of microfibrils sectioned mostly perpendicular to their axes. An enlargement of such a layer is seen in Figure 2A where the section of each microfibril is seen as a sharp-edged and nearly square shape with lateral dimensions in the order of 20 nm. When tested by microdiffraction, an area such as that indicated by the arrow in Figure 1A gives the electron diffraction diagram displayed in the inset. The diagram, which consists of 8 strong arcs, is similar to those published earlier by Revol and Goring (1983), Sugiyama *et al.* (1985). This diagram confirms the uniplanar-axial orientation (defined according to the classification of Heffelfinger and Burton, 1960) of the cellulose microfibrils within each cell wall lamella. It also confirms the statistical distribution of the polarity of the cellulose microfibrils within small domains of a given cell wall layer.

Figures 1B and 2B correspond to a cross section of *Valonia* III_I. In these pictures, the cellulose microfibrils which are perpendicular to the plane of observation are seen in black in Figure 1B and in white in Figure 2B. Each microfibril is still individualized and possesses roughly a size similar to those found in the initial *Valonia*. Remarkably, the shape of the sections

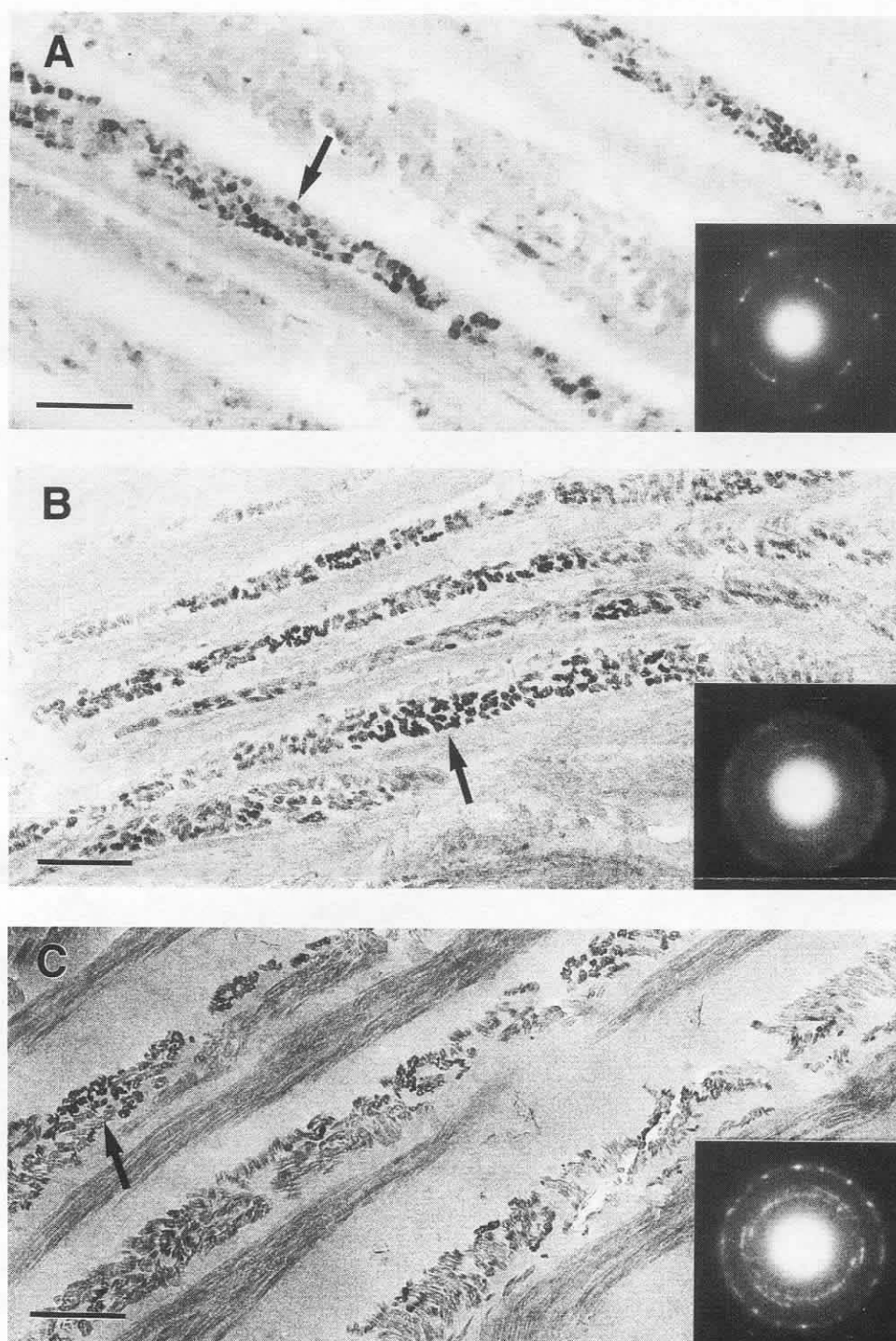


Fig. 1. Diffraction contrast electron micrograph of *Valonia ventricosa* cell wall sections with one of the main microfibrillar orientations perpendicular to the plane of observation. Scale bar 200 nm. **1A**: Initial *Valonia* I cell wall; **1B**: *Valonia* III; **1C**: Regenerated *Valonia* I; Insets: typical micro area electron diffraction of an area indicated by the arrow where the microfibrils are in cross-section.

is no longer square but adopts convoluted contours, with holes and cracks sometimes visible as far as their center. This is due to the successive action of massive intracrystalline swelling in ethylenediamine, followed by shrinkage during the washing step in methanol which removes the swelling agent. This may be compared with Figure 2A where each microfibril is a monocrystal and, as such, appears as a continuous

white area; this is no longer the case in Figure 2B, where a series of very small crystalline fragments, between 2 to 3 nm in width, are seen as white dots within each microfibril trace. The microdiffraction pattern (inset in Figure 1B) is weak, denoting a substantial loss of crystallinity. Continuous rings at 0.52 nm (medium intensity) and 0.75 nm (very weak intensity) are observed here as opposed to the arced pattern ob-

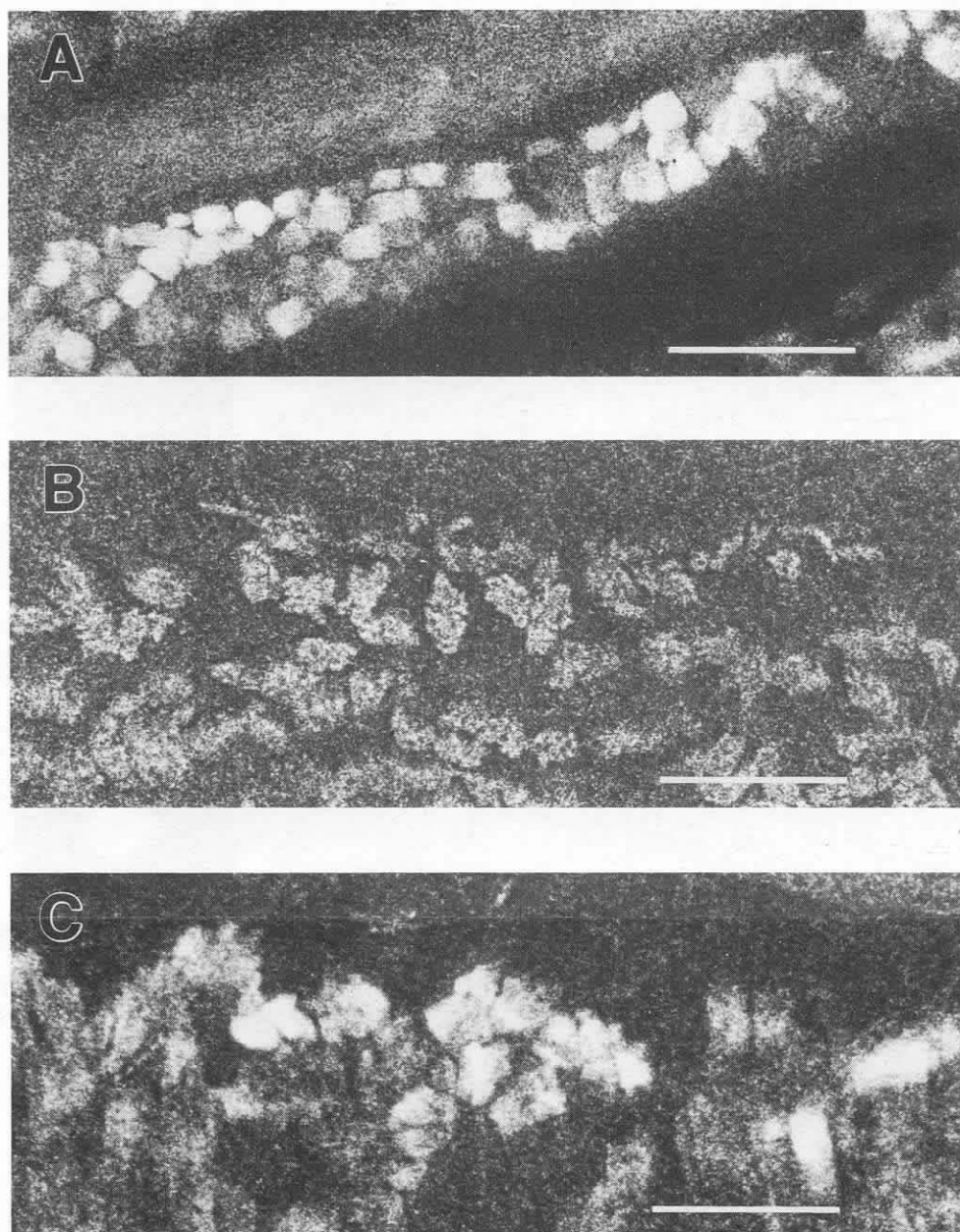


Fig. 2. A higher magnification of the micrographs in Fig. 1 printed in reverse contrast. Scale bar: 100 nm.

served in Figure 1A, clearly indicating the disappearance of the uniplanar-axial orientation of the cellulose crystals within the cell wall layers. The fragmentation noted in Figures 1B and 2B is consistent with such observed changes in crystallinity and orientation.

Figures 1C and 2C represent a cross section of a regenerated *Valonia* sample. In these Figures, as mentioned before, the tight stacking of the microfibril layers is lost and for this reason, only a small percentage of cellulose microfibrils are seen perpendicular to the plane of observation. The sections of the microfibrils also have convoluted contours and resemble closely those shown in Figures 1B and 2B, but the domains exhibiting diffraction contrast, i.e. the crystals themselves within the microfibrils sections, are mark-

edly increased. These domains, however, attain neither the perfection nor the size of those of the initial samples as in Figure 2A.

The selected-area-diffraction diagram of a well-oriented domain, such as the one indicated by the arrow (inset in Figure 1C), displays three sharp dotted rings with no specific orientation. The sharpness of the diffraction spots indicates that a substantial recrystallization took place during the hydrothermal treatment leading to regenerated *Valonia* I; this recrystallization, however, is not sufficient to restore the crystalline perfection and unique initial uniplanar-axial orientation, which was irreversibly lost when native cellulose was converted into the cellulose III₁ polymorph.

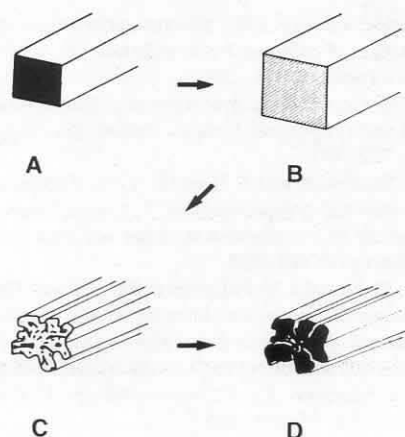


Fig. 3. Schematic representation of a *Valonia* microfibril undergoing swelling in ethylenediamine. **3A**: Initial *Valonia* I microfibril, consisting of one crystal represented in black. **3B**: Swollen microfibril after uptake of one ethylenediamine molecule per glucose residue. **3C**: Shrunken microfibril of *Valonia* III₁, resulting from the washing in methanol of a swollen microfibril as in Fig. 3B. The microfibril adopts a convoluted contour and is made of small crystalline domains (in black). **3D**: Microfibril as in 3C but after the hydrothermal treatment leading to regenerated *Valonia* I. The convoluted contour is maintained, but the crystalline domains (in black) are larger than in Fig. 3C.

Discussion

Based on these results, we present in Figure 3 the following schematic rationale for the crystalline distortion and fragmentation which develops from the so-called "reversible" transformation cellulose I cellulose III₁. During the intra-crystalline swelling of cellulose with ethylenediamine, each cellulose unit cell will accept 4 ethylenediamine molecules (Lee *et al.* 1984), resulting in an increase of the *a* and *b* cell parameters of the unit cell while *c* (the chain axis parameter) remains unaffected and γ (the monoclinic angle) is slightly modified. Overall, the surface of the unit cell base plane is roughly multiplied by a factor of 2 during swelling. Consequently, the cross-section surface of a typical *Valonia* microfibril (Figure 3A) is also multiplied by a factor of 2 (Figure 3B). This expansion destroys the unique inner cohesion of the microfibril that was created during its biogenesis. When the swelling agent is washed away, the microfibril is converted into cellulose III₁ as it shrinks back to a section, whose surface is close to that of the initial sample, according to the de-swelling process and the re-crystallization mechanism (Figure 3C). This is inevitably a poorly coordinated process leading to imperfect chain to chain registration, and hence to distortions and polycrystalline microfibrils such as those seen in Figures 1B and 2B. In the microfibril, which at this step adopts a convoluted contour, the initial uniplanar-axial orientation is irreversibly lost. The next transformation, which is reversion to cellulose I (Figure 3D) does not involve the transport of a swelling agent in and out of

the crystalline unit cell but involves only a small re-shuffling of the cellulose chains. This explains why the distorted microfibrils of cellulose III₁ and regenerated cellulose I have similar shape and cross-sections (Figures 3C and 3D). The increase in size of the crystals when changing from cellulose III₁ to cellulose I may be explained by the plasticization effect occurring in the presence of heat and water. This recrystallization could also be explained by a more favorable packing of the cellulose molecules into the cellulose I unit cell.

The present data are in agreement with our previous study (Roche and Chanzy 1981) where *Valonia* III₁ and regenerated *Valonia* I microfibrils parallel to the supporting film had been observed. From this earlier study, substantial subfibrillation of the initial microfibrils was one of the explanations for the observed negatively stained images. The present work, which takes advantage of superior diffraction contrast TEM pictures on transverse sections of cellulose microfibrils, leads to higher resolution images of the cellulose crystal. Remarkably, these pictures show that a subfibrillation of the microfibrils may not be as frequent as we initially thought. In fact, the modified microfibrils in *Valonia* III₁ and regenerated *Valonia* I are better described as being distorted, with grooves and ridges running along their length. As a result, in the previous study, the negative stain accumulated in these grooves created the false impression that the microfibrils were split into smaller sub-fibrils.

Our results help to understand why ethylenediamine treatment increases markedly the external surface area of the crystalline cellulose microfibrils and consequently their accessibility. With cellulose III₁, this surface increase coincides with a substantial decrystallization, making it much more reactive or "activated" (Herrick 1983) than either the initial native samples or even the regenerated cellulose I specimen.

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